Effect of BmDNV1 infection on the biochemical parameters in Densonucleosis type1 resistant and susceptible breeds of silkworm, *Bombyx mori* L.

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Abstract: Among silkworm diseases, viral diseases are the major and most common which damages the cocoon production. Viral flacherie is caused by BmIFV, BmDNV1 and BmDNV2. Resistant and susceptible silkworm breeds against BmDNV1 were identified among the Indian Germ plasm stock. The changes in major organic constituents *viz*., total protein, carbohydrate and lipid content during the progressive infection of BmDNV1 in susceptible and resistant breeds were estimated as per the standard methods. As the larval age increases, there was a consistent increase in the level of organic constituents *viz*., protein and carbohydrates in both control and treated batches, but the level of increase in treated batches were markedly less when compared to the respective control batches. After inoculation with BmDNV1, there was a significant decrease in total protein content in haemolymph and mid gut tissue in susceptible breeds when compared with control batches. In resistant breeds, there was a significant decrease in total protein content up to 4 day post inoculation (PI) but subsequently, the decrease was less compared to the respective controls. There was a slight decrease in total carbohydrate content in haemolymph and mid gut tissue in susceptible and resistant breeds. In case of lipid content, there was a significant increase in haemolymph and mid gut tissues in susceptible breeds and in resistant breeds the changes were not found significant. In susceptible silkworm breeds, the biochemical changes clearly indicated that the BmDNV1 infection depletes the major organic constituents *viz*., total protein and total carbohydrate contents which are the major sources energy. This depletion in these contents resulted in stunted growth in infected silkworms.

Key words: Silkworm; *Bombyx mori*; BmDNV1; total protein; total carbohydrate; total lipid; resistant breeds; susceptible breeds

1 INTRODUCTION

Sustainability of sericulture depends upon successful realization of cocoon crop throughout the year. In a tropical country like India, the success of silkworm rearing depends upon the protection of crop from the disease causing pathogens. The viral diseases in particular pose a major problem to sericulture as they account for almost 70% loss among various diseases. Non-occluded viruses *viz*., BmIFV, BmDNV1 and BmDNV2 cause viral flacherie in silkworm. The prevalence of the disease in India was recorded and a limited survey indicated its prevalence in 22.37% crops. The larvae infected with BmDNV1 show reduction in size, growth, body weight and flaccidity of body as the major symptoms followed by death (Watanabe and Kurihara, 1988). One of the most preferred approaches in management of diseases is to develop breeds resistant to diseases or the avoidance of extreme susceptibility to diseases. Sen *et al*. (2004) identified BmDNV1 resistant and susceptible breeds from Indian Germ plasm stock. The inheritances of resistance/susceptibility have been understood. However, the impact of BmDNV1 infection on the biochemical parameters of the resistant and susceptible breeds is not worked out. Hence, in the present study the impact of BmDNV1 infection on biochemical parameters was undertaken in two resistant and two susceptible silkworm breeds.

2 MATERIALS AND METHODS

BmDNV1 inoculum was received from Silkworm Pathology Section, C.S.R. & T. I., Mysore, India and the dilution of 10^{-1} (10 % gut homogenate) was

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utilized for the present study.

2.1 Selection of silkworm breeds and inoculation of BmDNV1

Based on the earlier works (Ratna Sen *et al*., 2004) two resistant breeds *viz*., H330 and C. nichi and two susceptible breeds *viz*., CSR2 and Pure Mysore (PM) were selected for the present study. These breeds were received from Silkworm Breeding Laboratory, CSR&TI, Mysore and were reared following the standard methods (Datta, 1992). To study the impact of BmDNV1 infection on biochemical parameters, fourth instar larvae of all the four breeds were divided into two batches. The first batch was inoculated with BmDNV1 (0.5 mL of 10^{-1} inoculum/100 larvae). The other batch was kept as the control without any inoculation. The treatment and control batches had three replications of 200 larvae. The larvae were reared till cocooning.

2.2 Collection of samples

Haemolymph from larvae of different treatment and control batches was collected on 0, 2, 4, 6, 8 and 10 days post inoculation (PI). To collect haemolymph larval proleg was cut and collected the haemolymph in eppendorf tubes containing a speck of phenylthiourea. The collected haemolymph was stored at 5℃ for further estimation of different organic constituents. The mid gut tissue from the larvae of different treatments and control batches was collected on 0, 2, 4, 6, 8 and 10 days PI and homogenized in sterilized distilled water to obtain 1% tissue homogenate. The homogenates were stored at 5℃ for further estimation of different organic constituents.

2.3 Estimation of total proteins, total carbohydrates and total lipids

The total protein content in the haemolymph and mid gut homogenate was estimated by following the method of Lowry *et al*. (1951) and the values are expressed as mg/mL of haemolymph and mg/g wet weight of mid gut tissue. The total carbohydrate content in the haemolymph and mid gut homogenate was estimated by the phenol-sulphuric acid method described by Dubois *et al*. (1956) and the values are expressed as mg/mL haemolymph and mg/g wet weight of mid gut tissue. The total lipid content in the haemolymph and gut samples was estimated gravimetrically following the method of Folch *et al*. (1951) and the quantity of lipid present was expressed in mg/mL of haemolymph and mg/g wet weight of tissue.

3 RESULTS

The observations recorded on total protein content in haemolymph and mid gut tissue during the course of BmDNV1 infection in the susceptible and resistant breeds are presented in Table 1 and 2. In CS2 breed the haemolymph and mid gut protein increased steadily as the age of the larvae increases both in control and BmDNV1 treated batches. On the day of inoculation (1st day of IV instar before resumption) the haemolymph protein content was 9.54 mg/mL and increased by 10th day PI to 38.30 mg/mL in the control and 18.20 mg/mL in BmDNV1 treated batch. In the same way, protein content in the mid gut tissue was also increased. But the total protein content of the haemolymph and mid gut tissues was significantly low in case of BmDNV1 treated larvae when compared to the respective controls. The same trend was observed in the protein content of haemolymph and mid gut tissues of PM breed. In H330 and C. nichi (resistant breeds) also the haemolymph and mid gut protein increased steadily as the age of the larvae increases both in control and BmDNV1 treated batches. However, the total protein content of the haemolymph and mid gut tissue in BmDNV1 treated batches has significantly reduced up to 4 day PI and in later days (up to 10th day PI) the reduction was not significant.

Table 1 Total protein content in the tissues during the course of BmDNV1 infection in susceptible breeds of silkworm

Sl. no.	Breed	Tissue	Treatment	Protein content in the tissues					
				0 day	2 day	4 day	6 day	8 day	10 day
1	CSR2	Haemolymph (mg/mL)	Control	9.54 ± 0.32	15.54 ± 0.34	18.71 ± 0.51	25.70 ± 0.70	30.20 ± 0.21	38.30 ± 0.30
			Treatment	9.54 ± 0.32	12.23 ± 0.12	13.43 ± 0.25	17.25 ± 0.31	16.41 ± 0.15	18.20 ± 0.25
			'T' Value	0^{NS}	-13.90^{*}	-16.10^{*}	-19.11^{*}	-54.51^{*}	-88.30^{*}
		Mid gut (mg/g)	Control	19.33 ± 0.32	21.67 ± 0.07	26.65 ± 0.46	28.65 ± 0.59	39.58 ± 0.47	52.47 ± 0.62
			Treatment	19.33 ± 0.32	18.69 ± 0.28	21.08 ± 0.79	22.07 ± 0.56	28.10 ± 0.18	30.20 ± 1.95
			'T' Value	0^{NS}	-18.87^{*}	-10.54^{*}	-13.92^{*}	-54.93^{*}	-18.83^{*}
2	PM	Haemolymph (mg/mL)	Control	6.31 ± 0.07	12.96 ± 1.27	13.61 ± 0.30	19.20 ± 0.28	24.60 ± 0.18	26.50 ± 0.25
			Treatment	6.31 ± 0.07	11.02 ± 0.09	11.19 ± 0.22	12.21 ± 0.66	15.20 ± 0.17	16.20 ± 0.26
			'T' Value	0^{NS}	-2.64^{NS}	-7.91^{*}	-16.99^{*}	-66.13^{*}	-50.16^{*}
		Mid gut (mg/g)	Control	11.96 ± 0.59	18.41 ± 0.25	26.69 ± 0.25	36.83 ± 0.58	36.40 ± 0.38	41.40 ± 1.08
			Treatment	11.96 ± 0.59	15.20 ± 1.43	18.03 ± 0.79	20.25 ± 1.47	23.13 ± 0.10	25.10 ± 0.76
			'T' Value	0^{NS}	-3.83^{NS}	-18.06^{*}	-11.58^{*}	-49.81^{*}	-21.41^{*}

Values are mean ± *SD* of six observations; NS means non significant; * means significant at 1% level. The same for the following tables.

Table 2 Total protein content in the tissues during the course of BmDNV1 infection in Resistant breeds of silkworm

Sl. no.	Breed	Tissue	Treatment	Protein content in the tissues					
				0 day	2 day	4 day	6 day	8 day	10 day
1	H330	Haemolymph (mg/mL)	Control	11543 ± 0.45	15.84 ± 1.41	17.83 ± 0.70	23.50 ± 0.53	29.60 ± 0.51	35.20 ± 0.47
			Treatment	11.43 ± 0.45	14.20 ± 0.31	15.20 ± 0.70	22.00 ± 0.23	27.80 ± 0.57	33.00 ± 1.76
			't' Value	0^{NS}	-1.97^{NS}	-4.64^{NS}	-4.94^{NS}	-4.90^{NS}	-2.09^{NS}
		Mid gut (mg/g)	Control	17.43 ± 0.49	22.56 ± 0.52	29.50 ± 0.42	33.85 ± 0.15	35.15 ± 0.24	48.30 ± 0.54
			Treatment	17.43 ± 0.49	19.69 ± 0.42	25.62 ± 0.63	31.62 ± 1.30	33.26 ± 0.72	46.20 ± 0.58
			't' Value	0^{NS}	-7.48^{*}	-8.95^{*}	-2.95^{NS}	-4.32^{NS}	-0.61^{NS}
2	C. nichi	Haemolymph (mg/mL)	Control	07.43 ± 0.43	11.43 ± 0.27	13.28 ± 0.26	15.84 ± 0.54	22.80 ± 0.19	25.30 ± 0.23
			Treatment	07.43 ± 0.43	10.42 ± 0.53	11.51 ± 0.43	15.00 ± 2.63	21.70 ± 0.68	24.60 ± 0.66
			't' Value	0^{NS}	-2.07^{NS}	-6.15^{*}	-0.54^{NS}	-2.67^{NS}	-1.73^{NS}
		Mid gut (mg/g)	Control	11.43 ± 0.47	18.10 ± 0.28	22.60 ± 0.46	28.40 ± 0.42	32.60 ± 0.48	40.44 ± 0.44
			Treatment	11.43 ± 0.47	16.68 ± 0.45	19.20 ± 0.45	27.32 ± 0.41	31.59 ± 0.45	38.80 ± 0.63
			't' Value	0^{NS}	-4.59^{NS}	-9.13^{*}	-9.13^{*}	-2.67^{NS}	-3.71^{NS}

The changes in the total carbohydrate content in haemolymph and mid gut tissue during the course of BmDNV1 infection in the susceptible and resistant breeds are presented in Table 3 and 4. In susceptible and resistant breeds, as the age of the larvae increases (control and treated batches) the haemolymph and mid gut carbohydrate content increased steadily. In CSR2 control batches, the carbohydrate content in the haemolymph and mid gut tissues increased from 7.04 to 9.21 and 11.82 to 26.05 respectively as the larval age increases from 0 day to 10th day while in treated batches it was 7.04 to 7.90 and 11.82 to 20.32. It is observed that the carbohydrate content of the haemolymph and mid gut tissue has reduced in BmDNV1 treated batches compared to the respective controls. The carbohydrate content in the haemolymph and midgut tissue of PM breed also showed the same trend of CSR2 both in the treated and control batches. In H330 and C. nichi (resistant breeds), there was a decrease in carbohydrate content of the haemolymph and mid gut tissue in BmDNV1 treated batches compared to respective controls.

Table 3 Total carbohydrate content in the tissues during the course of BmDNV1 infection in susceptible breeds of silkworm

Sl. no.	Breed	Tissue	Treatment	Carbohydrate content in the tissues					
				0 day	2 day	4 day	6 day	8 day	10 day
1	CSR2	Haemolymph (mg/mL)	Control	7.04 ± 0.04	7.22 ± 0.01	7.38 ± 0.08	7.68 ± 0.02	8.27 ± 0.45	9.21 ± 0.27
			Treatment	7.04 ± 0.04	7.05 ± 0.1	7.10 ± 0.40	7.30 ± 0.40	7.60 ± 0.22	7.90 ± 0.08
			't' Value	0^{NS}	-3.09^{NS}	-1.22^{NS}	-1.63^{NS}	-2.31^{NS}	-8.00^{*}
		Mid gut (mg/g)	Control	11.82 ± 0.66	12.06 ± 0.96	12.27 ± 0.23	15.10 ± 0.37	20.30 ± 1.26	26.05 ± 0.40
			Treatment	11.82 ± 0.66	11.90 ± 0.11	12.00 ± 0.30	13.00 ± 0.35	16.10 ± 0.69	20.32 ± 1.01
			't' Value	0^{NS}	-0.29^{NS}	-1.24^{NS}	-7.13^{*}	-4.98^{*}	-9.17^{*}
2	PM	Haemolymph (mg/mL)	Control	5.61 ± 0.43	6.85 ± 0.35	7.80 ± 0.90	8.32 ± 0.66	8.83 ± 0.31	9.44 ± 0.91
			Treatment	5.61 ± 0.43	6.02 ± 1.05	6.82 ± 0.26	7.18 ± 0.07	7.50 ± 0.25	7.52 ± 2.20
			't' Value	0^{NS}	-1.27^{NS}	-1.81^{NS}	-2.73^{NS}	-5.80^{*}	-1.40^{NS}
		Mid gut (mg/g)	Control	13.11 ± 0.47	14.20 ± 0.82	14.80 ± 0.19	15.10 ± 0.23	16.80 ± 1.21	18.46 ± 0.53
			Treatment	13.11 ± 0.47	13.52 ± 0.48	13.90 ± 0.37	14.10 ± 0.36	14.90 ± 0.67	16.20 ± 0.65
			't' Value	0^{NS}	-1.21^{NS}	-3.75^{NS}	-4.12^{NS}	-2.38^{NS}	-4.68^{NS}

Table 4 Total carbohydrate content in the tissues during the course of BmDNV1 infection in Resistant breeds of silkworm

Sl. no.	Breed	Tissue	Treatment	Carbohydrate content in the tissues					
				0 day	2 day	4 day	6 day	8 day	10 day
1	H330	Haemolymph (mg/mL)	Control	6.84 ± 0.15	7.01 ± 0.25	7.42 ± 0.21	7.65 ± 0.91	8.10 ± 1.01	8.66 ± 0.66
			Treatment	6.84 ± 0.15	6.92 ± 0.19	7.13 ± 0.26	7.34 ± 1.14	7.73 ± 1.20	8.22 ± 0.40
			't' Value	0^{NS}	-0.50^{NS}	-1.15^{NS}	-0.51^{NS}	-0.24^{NS}	-0.98^{NS}
		Mid gut (mg/g)	Control	13.60 ± 0.60	14.26 ± 0.39	16.40 ± 0.10	17.09 ± 0.26	17.69 ± 0.26	18.84 ± 0.93
			Treatment	13.60 ± 0.60	13.92 ± 0.17	15.13 ± 0.93	16.54 ± 0.12	17.01 ± 0.12	17.85 ± 0.23
			't' Value	0^{NS}	-1.26^{NS}	-2.34^{NS}	-0.46^{NS}	-4.13^{NS}	1.79^{NS}
2	C. nichi	Haemolymph (mg/mL)	Control	5.22 ± 0.27	6.83 ± 1.01	7.45 ± 0.79	7.82 ± 0.61	8.44 ± 0.49	8.56 ± 0.28
			Treatment	5.22 ± 0.27	6.40 ± 0.38	6.85 ± 0.36	7.41 ± 0.22	8.24 ± 0.21	8.31 ± 0.23
			't' Value	0^{NS}	-0.77^{NS}	-1.20^{NS}	-1.20^{NS}	-0.60^{NS}	-1.21^{NS}
		Mid gut (mg/g)	Control	12.55 ± 0.25	12.90 ± 0.32	13.50 ± 0.20	14.60 ± 0.29	16.08 ± 0.10	18.56 ± 0.26
			Treatment	12.55 ± 0.25	12.64 ± 0.56	12.52 ± 0.64	13.72 ± 0.25	15.20 ± 0.24	17.61 ± 0.05
			't' Value	0^{NS}	-0.69^{NS}	-2.53^{NS}	-6.59^{*}	-5.83^{*}	-6.20^{*}

As the age of the larvae increases (IV instar to V instar) the total lipid content increased both in control and treated batches of susceptible and resistant breeds. The per cent changes in total lipid content over the control in the tissues during the course of BmDNV1 infection in susceptible and resistant breeds are presented in Fig. 1 and 2. The lipid content of the haemolymph and mid gut tissue in treated batches has significantly increased when compared to the respective controls. In CSR2, the per cent increase in the lipid content of the haemolymph from 0 day to 10th day was 0.0 to 10.67 and mid gut tissue was 0.0 to 23.92. In case of PM the per cent increase of lipid content in the haemolymph and mid gut tissue showed similar trend (Fig. 1). In the resistant breeds, H330 and C. nichi, the lipid content of the haemolymph and mid gut tissue has significantly increased up to 4 day of PI but further the increase was less when compared to the respective controls (Fig. 2).

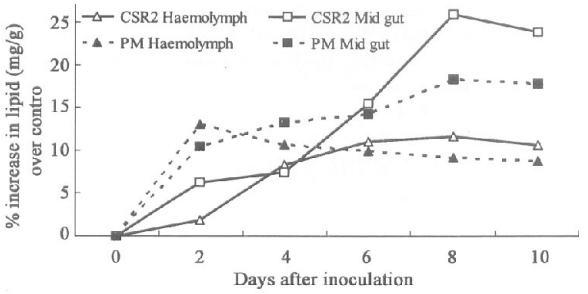

Fig. 1 Per cent changes over the control in total lipid content in the tissues during the course of BmDNV1 infection in susceptible breeds of silkworm

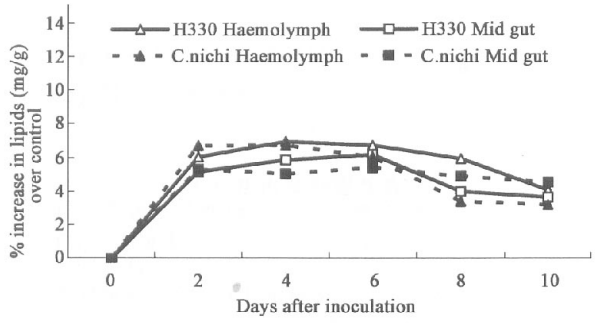

Fig. 2 Per cent changes over the control in total lipid content in the tissues during the course of BmDNV1 infection in resistant breeds of silkworm

4 DISCUSSION

Pathogenic infections are reported to induce biochemical and physiological alterations in insect tissues (Martignoni, 1964; Shigematsu and Noguchi, 1969). It is observed from the results of the present study that BmDNV1 is influencing the biochemical constituents such as protein, carbohydrate and lipid contents of silkworm. The influence of infection on haemolymph and mid gut total protein, carbohydrate and lipid level is well pronounced in BmDNV1 susceptible breeds. In resistant breeds the influence of BmDNV1 infection in haemolymph and mid gut protein, carbohydrate and lipid is insignificant and the levels were similar to the untreated control batches. In resistant breeds after the inoculation with BmDNV1 the protein contents decreased up to 4th day PI but picked up in later days and were on par with the control. The observations clearly indicate that BmDNV1 infection in silkworm influence the protein, carbohydrate and lipid content. But the influence is minimised in BmDNV1 resistant breeds. The morphological observations during the progressive infection of BmDNV1 in susceptible breeds (CSR2 and PM) indicated the clear symptoms of flacherie disease *viz*., reduction in larval size, growth, body weight and flaccidity. Larvae of the resistant breeds (H330 and C. nichi) have not exhibited symptoms of the disease and were observed to be normal.

The proteins are derivatives of high molecular weight polypeptides, which play a vital role in the formation of structure of different organs. The decrease in total protein content in haemolymph and mid gut tissue can be attributed to the disintegration of structural organization at sub cellular level. Another reason for the decrease in protein content may be either by activated proteolysis or impaired protein synthesis in the tissues during the infection. The growth and development of larvae always depends on the active synthesis of protein in the tissues (Engetmann, 1965; Tazima, 1978), the decreased protein content during BmDNV1 infection therefore could have inhibited the process of larval development. It is evident from the stunted growth of the larvae observed during BmDNV1 infection in susceptible breeds. There was a significant depletion in total carbohydrate content in haemolymph and mid gut tissue during the progressive infection of BmDNV1 in susceptible breeds (CSR2 and PM) compared to the control. But in the resistant breeds (H330 and C. nichi) there was a decrease in carbohydrate content up to 4 days of PI but afterwards the changes were minimum and the larvae recouped to the normal level. Carbohydrates serve as main source of energy to a number of insect species (Chino and Gilbert, 1965). As energy is a vital force in the biological system, a break down of organic constituents mainly carbohydrates is required to meet the energy under stress condition. The decreased carbohydrate levels in haemolymph and mid gut tissue can be attributed to excessive utilization of carbohydrate to meet the demand of energy to cope with BmDNV1 infection.

There was a significant increase in total lipid content in haemolymph and mid gut tissue during the progressive infection of BmDNV1 in susceptible breeds compared to the control. But in the resistant breeds, there was an elevation in the level of lipid content after BmDNV1 inoculation. However, the elevation was not significant. Lipids serve as a source of metabolic energy as well as essential for structural components of cells. They also play a role in the synthesis of viral envelopes (Bergold and Wellington, 1954) probably being responsible for the infectivity of virus (Yamamoto and Tanada, 1978). The results of the present study revealed that the amount of lipid in the haemolymph and mid gut tissue increased markedly in susceptible breeds when infected with BmDNV1. The marked increase in the lipid content of virus infected larvae had been attributed to the altered host lipid metabolism (Komano *et al*., 1966; Boctor, 1981). In resistant breeds the increase was marginal and in significant compared to the control.

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感染家蚕浓核病毒 BmDNV1 对家蚕抗性和敏感品种生化参数的影响

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摘要：在家蚕疾病中，病毒性疾病是危害茧产品的主要且最普遍的疾病。病毒性蚕软腐病是由 BmIFV, BmDNV1 和 BmDNV2 引起的。对印度种质库(Indian Germ plasm stock)中的 BmDNV1 有抗性和敏感的家蚕品种进行了鉴定。利用标准方法对在 BmDNV1 感染过程中抗性和敏感品系的主要有机成分的变化(包括总蛋白、碳水化合物和脂类)进行了检测。结果表明：随着幼虫年龄的增长，对照组和处理组中有机成分(即蛋白质和碳水化合物)的含量也随之增长，但是处理组的增长水平明显地低于各自对照组的增长水平。接种 BmDNV1 后，与对照组比较，敏感品种体内的血淋巴和中肠组织的总蛋白的量显著地下降。在抗性品种，接种 4 天后的总蛋白的量有了显著地下降，之后，下降水平小于各自的对照组。在抗性和敏感品种中，血淋巴和中肠组织中的总碳水化合物的量略有下降。在变化不显著的抗性和敏感品种中，血淋巴和中肠组织中的脂质的量有显著地提高。在敏感家蚕品种，生化变化清晰地显示：BmDNV1 感染消耗作为主要能量来源的总蛋白和碳水化合物。这些成分的损耗导致了被感染家蚕的生长受阻。

关键词：家蚕；家蚕浓核病毒；总蛋白；总碳水化合物；总脂类；抗性品系；敏感品系

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